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(54) Title: ZCYTOR7 CYTOKINE RECEPTOR

(57) Abstract

Novel cytokine receptor polypeptides, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed. The polypeptides comprise an extracellular domain of a cell–surface receptor that is expressed in kidneys, pancreas, prostate, adrenal cortex and nervous tissue. The polypeptides may be used within methods for detecting ligands that promote the proliferation and/or differentiation of these organs.

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ZCYTOR7 CYTOKINE RECEPTOR

BACKGROUND OF THE INVENTION

Cytokines are soluble proteins that influence the growth and differentiation of many cell types. Their receptors are composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains. For example, the receptor chains responsible for binding and/or transducing the effect of interferons (IFNs) are members of the type II cytokine receptor family (CRF2), based upon a characteristic 200 residue extracellular domain. The demonstrated *in vivo* activities of these interferons illustrate the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and cytokine antagonists.

SUMMARY OF THE INVENTION

The present invention fills this need by providing novel cytokine receptors and related compositions and methods. In particular, the present invention provides for an extracellular ligand-binding region of a mammalian Zcytor7 receptor, alternatively also containing either a transmembrane domain or both an intracellular domain and a transmembrane domain.

Within one aspect, the present invention provides an isolated polynucleotide encoding a ligand-

binding receptor polypeptide. The polypeptide comprises a sequence of amino acids selected from the group consisting of (a) SEQ ID NO:13, residues 30 through 250 of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b). Within one embodiment, the polypeptide comprises the amino acid sequence defined by SEQ ID NO: 13 or amino acid residues 30 through 250 of SEQ ID NO:2. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises a transmembrane domain. The transmembrane domain may comprise residues 251 through 274 of SEQ ID NO:2, or an allelic variant thereof. Within another embodiment, the polypeptide encoded by the isolated polynucleotide further comprises an intracellular domain, such as an intracellular domain comprising residues 275 through 553 of SEQ ID NO:2, or an allelic variant thereof. Within further embodiments, the polynucleotide encodes a polypeptide that comprises residues 1 through 553, 1 through 274, 1 through 250, 30 through 274 or 30 through 553 of SEQ ID NO:2. Within an additional embodiment, the polypeptide further comprises an affinity tag. Within a further embodiment, the polynucleotide is DNA. Also claimed are the isolated polypeptides encoded by these polynucleotides.

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding a ligand-binding receptor polypeptide, wherein the ligand-binding receptor polypeptide comprises a sequence of amino acids selected from the group consisting of: (i) residues 30 through 250 of SEQ ID NO:2; (ii) allelic variants of (i); and (iii) sequences that are at least 80% identical to (i) or (ii); and (c) a transcription terminator, wherein the promoter, DNA segment, and terminator are operably linked. The ligand-binding

receptor polypeptide may further comprise a secretory peptide, a transmembrane domain, a transmembrane domain and an intracellular domain, or a secretory peptide, a transmembrane domain and an intracellular domain.

Within a third aspect of the invention there is provided a cultured eukaryotic cell into which has been introduced an expression vector as disclosed above, wherein said cell expresses a receptor polypeptide encoded by the DNA segment. Within one embodiment, the cell further expresses a necessary receptor subunit which forms a functional receptor complex. Within another embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for proliferation.

Within a fourth aspect of the invention there is provided an isolated polypeptide comprising a sequence selected from the group consisting of (a) residues 30, a valine, through residue 250, a lysine, of SEQ ID NO:2; (b) allelic variants of (a); and (c) sequences that are at least 80% identical to (a) or (b), wherein said polypeptide is substantially free of transmembrane and intracellular domains ordinarily associated with hematopoietic receptors. Also claimed are polypeptides comprised of a sequence defined by residues 30, a valine, through residue 274, a tyrosine; and a polypeptide comprised of a sequence defined by residues 30, a valine, through residue 553 an asparagine. Also claimed are the polypeptides and polynucleotides defined by the sequences of SEQ ID NOs: 13 - 60.

Within a further aspect of the invention there is provided a chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of a ligand binding

domain of a receptor polypeptide selected from the group consisting of (a) a receptor polypeptide as shown in SEQ ID NO:2; (b) allelic variants of SEQ ID NO:2; and (c) receptor polypeptides that are at least 80% identical to (a) or (b). The second portion of the chimeric polypeptide consists essentially of an affinity tag. Within one embodiment the affinity tag is an immunoglobulin F_C polypeptide. The invention also provides expression vectors encoding the chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

The present invention also provides for an isolated polynucleotide encoding a polypeptide selected from a group defined SEQ ID NO:2 consisting of residues 1 through 250, residues 1 through 274, residues 1 through 553, residues 2 through 250, residues 2 through 274, residues 2 through 553, residues 251 through 274, residues 251 through 553 and residues 275 through 553. Also claimed are the isolated polypeptide expressed by these polynucleotides.

The invention also provides a method for detecting a ligand within a test sample, comprising contacting a test sample with a polypeptide as disclosed above, and detecting binding of the polypeptide to ligand in the sample. Within one embodiment the polypeptide further comprises transmembrane and intracellular domains. The polypeptide can be membrane bound within a cultured cell, wherein the detecting step comprises measuring a biological response in the cultured cell. Within another embodiment, the polypeptide is immobilized on a solid support.

Within an additional aspect of the invention there is provided an antibody that specifically binds to a

polypeptide as disclosed above, as well as an anti-idiotypic antibody which binds to the antigen-binding region of an antibody to Zcytor7.

In still another aspect of the present invention, polynucleotide primers and probes are provided which can detect mutations in the Zcytor7 gene. The polynucleotide probe should at least be 20-25 bases in length, preferably at least 50 bases in length and most preferably about 80 to 100 bases in length. In addition to the detection of mutations, these probes can be used to discover the Zcytor7 gene in other mammalian species. The probes can either be positive strand or anti-sense strands, and they can be comprised of DNA or RNA.

The present invention is further comprised of polypeptides comprised of a subsequence of SEQ ID NO:2. The subsequence is comprised of from 20 to 552 amino acids residues from SEQ ID NO:2 which are antigenic and against which a warm blooded animal is able to produce antibodies against when the polypeptide is injected into the animal and said antibodies are able to bind to the polypeptide of SEQ ID NO: 2. Preferably, the subsequence is a subsequence of SEQ ID NO: 13. Preferably, the subsequence contains at least 50 amino acids from SEQ ID NO: 2.

These and other aspects of the invention will become evident upon reference to the following detailed description and the attached drawing.

DETAILED DESCRIPTION OF THE INVENTION

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation

arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems.

"Operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in the promoter and proceeds through the coding segment to the terminator.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural

sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules.

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

The term "receptor" is used herein to denote a cell-associated protein, or a polypeptide subunit of such a protein, that binds to a bioactive molecule (the "ligand") and mediates the effect of the ligand on the cell. Binding of ligand to receptor results in a conformational change in the receptor (and, in some cases, receptor multimerization, i.e., association of identical or different receptor subunits) that causes interactions between the effector domain(s) and other molecule(s) in the cell. These interactions in turn lead to alterations in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, cell proliferation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. The term "receptor polypeptide" is used to denote complete receptor polypeptide chains and portions thereof, including isolated functional domains (e.g., ligand-binding domains).

A "secretory signal sequence" is a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which

it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues, such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are produced by proteolysis or translated from alternatively spliced mRNAs. Receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a protein having the structure of a cytokine receptor, including the conserved WSXWS motif (SEQ ID NO:10). Analysis of the tissue distribution of the mRNA corresponding to this novel DNA showed that mRNA level was highest in pancreas, prostate, kidney and adrenal cortex followed by lower levels in testis, stomach, adrenal medulla and thymus. The receptor has been designated "Zcytor7".

Cytokine receptors subunits are characterized by a multi-domain structure comprising a ligand-binding domain and an effector domain that is typically involved in signal transduction. Multimeric cytokine receptors

include homodimers (e.g., PDGF receptor αα and ββ isoforms, erythropoietin receptor, MPL [thrombopoietin receptor], and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor αβ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of their structures and functions. Class I hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif (SEQ ID NO:10). Additional domains, including protein kinase domains; fibronectin type III domains; and immunoglobulin domains, which are characterized by disulfide-bonded loops, are present in certain hematopoietic receptors. Cytokine receptor structure has been reviewed by Urdal, *Ann. Reports Med. Chem.* 26:221-228 (1991) and Cosman, *Cytokine* 5:95-106 (1993). It is generally believed that under selective pressure for organisms to acquire new biological functions, new receptor family members arose from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation and identification of additional family members.

Cell-surface cytokine receptors are further characterized by the presence of additional domains. These receptors are anchored in the cell membrane by a transmembrane domain characterized by a sequence of

hydrophobic amino acid residues (typically about 21-25 residues), which is commonly flanked by positively charged residues (Lys or Arg). On the opposite end of the protein from the extracellular domain and separated from it by the transmembrane domain is an intracellular domain.

The novel receptor of the present invention, Zcytor7, is a class II cytokine receptor. These receptors usually bind to four-helix-bundle cytokines. Interleukin-10 and the interferons have receptors in this class (e.g., interferon-gamma alpha and beta chains and the interferon-alpha/beta receptor alpha and beta chains). Class II cytokine receptors are characterized by the presence of one or more cytokine receptor modules (CRM) in their extracellular domains. The CRMs of class II cytokine receptors are somewhat different than the better known CRMs of class I cytokine receptors. While the class II CRMs contain two type-III fibronectin-like domains, they differ in organization. In particular, they contain two WSXWS (SEQ ID NO: 10) motifs, one in each fibronectin III-like domain. These WSXWS (SEQ ID NO: 10) motifs, however, are less conserved than those found in class I CRMs.

Zcytor7, like all known class II receptors except interferon-alpha/beta receptor alpha chain, has only a single class II CRM in its extracellular domain. Zcytor7 appears to be a receptor for a helical cytokine of the interferon/IL-10 class. Using the Zcytor7 receptor we can identify ligands and additional compounds which would be of significant therapeutic value. Furthermore, the extracellular portion of Zcytor7 extending from residue 30, a valine, through residue 250 of SEQ ID NO: 2 can be expressed and used as a soluble receptor to down-regulate the effects of the ligand of Zcytor7.

As was stated above, Zcytor7 was initially identified by the overall homology to CRF2-4, an orphan Class II cytokine receptor. See Lutfalla G. *et al. Genomics, 16:* 366-373 (1993). Analysis of a human cDNA clone encoding Zcytor7 (SEQ ID NO:1) revealed an open reading frame encoding 553 amino acids (SEQ ID NO:2) comprising an extracellular ligand-binding domain of approximately 221 amino acid residues (residues 30-250 of SEQ ID NO:2), a transmembrane domain of approximately 24 amino acid residues (residues 251-274 of SEQ ID NO:2), and an intracellular domain of approximately 279 amino acid residues (residues 275-553 of SEQ ID NO:2). Those skilled in the art will recognize that these domain boundaries are approximate and are based on alignments with known proteins and predictions of protein folding. Deletion of residues from the ends of the domains is possible.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1 or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typical stringent conditions are those in which the salt concentration is at least about 0.02 M at pH 7 and the temperature is at least about 60°C. As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for isolating DNA and RNA are well known in the art. It is generally preferred to isolate RNA from pancreas or prostate tissues although cDNA can also be prepared using RNA from other tissues or isolated as genomic DNA. Total RNA can be prepared using guanidine HCl extraction

followed by isolation by centrifugation in a CsCl gradient (Chirgwin *et al.*, *Biochemistry 18*:52-94 (1979). Poly (A)$^+$ RNA is prepared from total RNA using the method of Aviv and Leder, *Proc. Natl. Acad. Sci. USA 69*:1408-1412 (1972). Complementary DNA (cDNA) is prepared from poly(A)$^+$ RNA using known methods. Polynucleotides encoding Zcytor7 polypeptides are then identified and isolated by, for example, hybridization or PCR.

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NOS:1 and 2 represent single alleles of the human Zcytor7 receptor. Allelic variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures.

The present invention further provides counterpart receptors and polynucleotides from other species ("species orthologs"). Of particular interest are Zcytor7 receptors from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate receptors. Species orthologs of the human Zcytor7 receptor can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses the receptor. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A receptor-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No.

4,683,202), using primers designed from the sequences disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to the receptor. Similar techniques can also be applied to the isolation of genomic clones.

The present invention also provides isolated receptor polypeptides that are substantially homologous to the receptor polypeptide of SEQ ID NO: 2. By "isolated" is meant a protein or polypeptide that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. The term "substantially homologous" is used herein to denote polypeptides having 50%, preferably 60%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2,. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2. Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al.*, *Bull. Math. Bio. 48*: 603-616 (1986) and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA 89*:10915-10919 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blossom 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 1 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Table 1

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Substantially homologous proteins and polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 2) and other substitutions that do not significantly affect the folding or activity of the protein or polypeptide. Also claimed are deletions of SEQ ID NO:2, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification (an affinity tag), such as a poly-histidine tract, protein A, Nilsson *et al.*, *EMBO J.* *4*:1075 (1985); Nilsson *et al.*, *Methods Enzymol.* *198*:3 (1991), glutathione S transferase, Smith and Johnson, *Gene* *67*:31 (1988), or other antigenic epitope or binding domain. See, in general Ford *et al.*, *Protein Expression and Purification* *2*: 95-107, 1991. DNAs encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, NJ).

Table 2

Conservative amino acid substitutions	
Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine

TABLE 2, continued

Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

Essential amino acids in the receptor polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis, Cunningham and Wells, *Science 244*, 1081-1085 (1989); Bass *et al.*, *Proc. Natl. Acad. Sci. USA 88*:4498-4502 (1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity (e.g., ligand binding and signal transduction) to identify amino acid residues that are critical to the activity of the molecule. Sites of ligand-receptor interaction can also be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labeling. See, for example, de Vos *et al.*, *Science 255*:306-312 (1992); Smith *et al.*, *J. Mol. Biol. 224*:899-904 (1992); Wlodaver *et al.*, *FEBS Lett. 309*:59-64 (1992). The identities of essential amino acids can also be inferred from analysis of homologies with related receptors.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer, *Science 241*:53-57 (1988) or Bowie and Sauer, *Proc. Natl. Acad. Sci. USA 86*:2152-2156 (1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman *et al.*, *Biochem. 30*:10832-10837 (1991); Ladner *et al.*, U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis, Derbyshire *et al.*, *Gene 46*:145 (1986); Ner *et al.*, *DNA* 7:127 (1988).

Mutagenesis methods as disclosed above can be combined with high-throughput screening methods to detect activity of cloned, mutagenized receptors in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding fragments) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed above, one of ordinary skill in the art can prepare a variety of polypeptides that are substantially homologous to residues 30 to 250 of SEQ ID NO:2 or allelic variants thereof and retain the ligand-binding properties of the wild-type receptor. Such polypeptides may include additional amino

acids from an extracellular ligand-binding domain of a Zcytor7 receptor as well as part or all of the transmembrane and intracellular domains. Such polypeptides may also include additional polypeptide segments as generally disclosed above.

Polynucleotides, generally a cDNA sequence, of the present invention encode the above-described polypeptides. A cDNA sequence which encodes a polypeptide of the present invention is comprised of a series of codons, each amino acid residue of the polypeptide being encoded by a codon and each codon being comprised of three nucleotides. The amino acid residues are encoded their respective codons as follows.

Alanine (Ala) is encoded by GCA, GCC, GCG or GCT;

Cysteine (Cys) is encoded by TGC or TGT;

Aspartic acid (Asp) is encoded by GAC or GAT;

Glutamic acid (Glu) is encoded by GAA or GAG;

Phenylalanine (Phe) is encoded by TTC or TTT;

Glycine (Gly) is encoded by GGA, GGC, GGG or GGT;

Histidine (His) is encoded by CAC or CAT;

Isoleucine (Ile) is encoded by ATA, ATC or ATT;

Lysine (Lys) is encoded by AAA, or AAG;

Leucine (Leu) is encoded by TTA, TTG, CTA, CTC, CTG or CTT;

Methionine (Met) is encoded by ATG;

Asparagine (Asn) is encoded by AAC or AAT;

Proline (Pro) is encoded by CCA, CCC, CCG or CCT;

Glutamine (Gln) is encoded by CAA or CAG;

Arginine (Arg) is encoded by AGA, AGG, CGA, CGC, CGG or CGT;

Serine (Ser) is encoded by AGC, AGT, TCA, TCC, TCG or TCT;

Threonine (Thr) is encoded by ACA, ACC, ACG or ACT;

Valine (Val) is encoded by GTA, GTC, GTG or GTT;

Tryptophan (Trp) is encoded by TGG; and

Tyrosine (Tyr) is encoded by TAC or TAT.

It is to be recognized that according to the present invention, when a cDNA is claimed as described above, it is understood that what is claimed are both the sense strand, the anti-sense strand, and the DNA as double-stranded having both the sense and anti-sense strand annealed together by their respective hydrogen bonds. Also claimed is the messenger RNA (mRNA) encodes the polypeptides of the present invention, and which mRNA is encoded by the above the above-described cDNA. A messenger RNA (mRNA) will encode a polypeptide using the same codons as those defined above, with the exception that each thymine nucleotide (T) is replaced by a uracil nucleotide (U).

The receptor polypeptides of the present invention, including full-length receptors, receptor fragments (e.g. ligand-binding fragments), and fusion polypeptides can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory

Press, Cold Spring Harbor, NY (1989), and Ausubel *et al.*, *ibid.*

The polynucleotides of the present invention can be synthesized using DNA synthesizers. Currently the method of choice is the phosphoramidite method.

An initial set of overlapping, complementary oligonucleotides, each of which is between 20 to 60 nucleotides long is first made. Each internal section of the gene has complementary 3' and 5' terminal extensions that are designed to base pair precisely with an adjacent section. Thus, after the gene is assembled, the only remaining requirement to complete the process is sealing the nicks along the backbones of the two strands with T4 DNA ligase. Furthermore, terminal sequences that facilitate insertion into a restriction endonuclease sites of a cloning vector and other sequences should also be added that contain signals for the proper initiation and termination of transcription and translation.

An alternative way to prepare a full-size gene is to synthesize a specified set of overlapping oligonucleotides (40 to 100 nucleotides). The duplex is completed and the gaps filled by enzymatic DNA synthesis with *E. coli* DNA polymerase I. This enzyme uses the 3'-hydroxyl groups as replication initiation points and the single-stranded regions as templates. After the enzymatic synthesis is completed, the nicks are sealed with T4 DNA ligase. For larger genes (≥ 1,000 base pairs), the complete gene sequence is usually assembled from double-stranded fragments that are each put together by joining four to six overlapping oligonucleotides (20 to 60 bp each). If there is a sufficient amount of the double-stranded fragments after each synthesis and annealing step, they are simply joined to one another. Otherwise,

each fragment is cloned into a vector to amplify the amount of DNA available. In both cases, the double-stranded constructs are sequentially linked to one another to form the entire gene sequence. See Glick, Bernard R. and Jack J. Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, (ASM Press, Washington, D.C. 1994), Itakura, K. *et al.* Synthesis and use of synthetic oligonucleotides. *Annu. Rev. Biochem. 53* : 323-356 (1984), and Climie, S. *et al.* Chemical synthesis of the thymidylate synthase gene. *Proc. Natl. Acad. Sci. USA 87* :633-637 (1990).

A DNA sequence encoding a Zcytor7 receptor polypeptide can then be operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a Zcytor7 receptor polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of the receptor, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is joined to the Zcytor7 DNA sequence in the correct

reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Cultured mammalian cells are preferred hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection, Wigler *et al.*, *Cell* 14:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981: Graham and Van der Eb, *Virology* 52:456 (1973), electroporation, Neumann *et al.*, *EMBO J.* 1:841-845 (1982), DEAE-dextran mediated transfection, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY (1987), and liposome-mediated transfection, Hawley-Nelson *et al.*, *Focus* 15:73 (1993); *Ciccarone et al.*, *Focus* 15:80 (1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham *et al.*, *J. Gen. Virol.* 36:59-72 (1977) and Chinese hamster ovary (*e.g.* CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, *e.g.*, U.S. Patent No. 4,956,288. Other suitable promoters include those from

metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222; Bang et al., U.S. Patent No. 4,775,624; and WIPO publication WO 94/06463. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, *J. Biosci. (Bangalore) 11*:47-58 (1987).

Fungal cells, including yeast cells, and particularly cells of the genus *Saccharomyces*, can also be used within the present invention, such as for producing receptor fragments or polypeptide fusions. Methods for transforming yeast cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* *132*:3459-3465 (1986) and Cregg, U.S. Patent No. 4,882,279. Aspergillus cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming Neurospora are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

Within one aspect of the present invention, a novel receptor is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand, as well as agonists and antagonists of the natural ligand. To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

Mammalian cells suitable for use in expressing Zcytor7 receptors and transducing a receptor-mediated signal include cells that express other receptor subunits which may form a functional complex with Zcytor7. These subunits may include those of the interferon receptor family or of other class II or class I cytokine receptors. It is also preferred to use a cell from the same species as the receptor to be expressed. Within a preferred

embodiment, the cell is dependent upon an exogenously supplied hematopoietic growth factor for its proliferation. Preferred cell lines of this type are the human TF-1 cell line (ATCC number CRL-2003) and the AML-193 cell line (ATCC number CRL-9589), which are GM-CSF-dependent human leukemic cell lines. In the alternative, suitable host cells can be engineered to produce the necessary receptor subunit or other cellular component needed for the desired cellular response. For example, the murine cell line BaF3, Palacios and Steinmetz, *Cell 41*: 727-734 (1985); Mathey-Prevot *et al.*, *Mol. Cell. Biol. 6*: 4133-4135 (1986) or a baby hamster kidney (BHK) cell line can be transfected to express the necessary β subunit (also known as KH97) as well as a Zcytor7 receptor. The latter approach is advantageous because cell lines can be engineered to express receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. In the alternative, species orthologs of the human receptor cDNA can be cloned and used within cell lines from the same species, such as a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as GM-CSF, can thus be engineered to become dependent upon a Zcytor7 ligand.

Cells expressing functional receptor are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the detection of a biological response in a target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the metabolic breakdown of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Mosman, *J. Immunol. Meth.* 65: 55-63 (1983). An

alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, or SRE (see, e.g., Shaw *et al.*, *Cell* 56:563-572 (1989). A preferred such reporter gene is a luciferase gene, de Wet *et al.*, *Mol. Cell. Biol.* 7:725 (1987). Expression of the luciferase gene is detected by luminescence using methods known in the art, *e.g.*, Baumgartner *et al.*, *J. Biol. Chem.* *269*:29094-29101 (1994); Schenborn and Goiffin, *Promega Notes* *41*:11 (1993). Luciferase activity assay kits are commercially available from, for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian expression vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools, re-transfection, subculturing, and re-assay of positive cells to isolate a cloned cDNA encoding the ligand.

A natural ligand for the Zcytor7 receptor can also be identified by mutagenizing a cell line expressing the receptor and culturing it under conditions that select for autocrine growth. See WIPO publication WO 95/21930. Within a typical procedure, BaF3 cells expressing Zcytor7 and the necessary additional subunits are mutagenized, such as with 2-ethylmethanesulfonate (EMS). The cells are then allowed to recover in the presence of IL-3, then

transferred to a culture medium lacking IL-3 and IL-4. Surviving cells are screened for the production of a Zcytor7 ligand, such as by adding soluble receptor to the culture medium or by assaying conditioned media on wild-type BaF3 cells and BaF3 cells expressing the receptor.

An additional screening approach provided by the present invention includes the use of hybrid receptor polypeptides. These hybrid polypeptides fall into two general classes. Within the first class, the intracellular domain of Z-Cytor7, comprising approximately residues 275 to 553 of SEQ ID NO:2, is joined to the ligand-binding domain of a second receptor. It is preferred that the second receptor be a hematopoietic cytokine receptor, such as mpl receptor, Souyri *et al.*, *Cell 63*: 1137-1147 (1990). The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA construct encoding the hybrid receptor is then inserted into a host cell. Cells expressing the hybrid receptor are cultured in the presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by Zcytor7 while using readily available ligands. This system can also be used to determine if particular cell lines are capable of responding to signals transduced by Zcytor7. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain of Zcytor7 (approximately residues 30 to 250 of SEQ ID NO:2) with an intracellular domain of a second receptor, preferably a hematopoietic cytokine receptor, and a transmembrane domain. Hybrid receptors of this second class are expressed in cells known to be capable of responding to signals transduced by the second receptor. Together, these two classes of hybrid receptors enable the

identification of a responsive cell type for the development of an assay for detecting a Zcytor7 ligand.

Cells found to express the ligand are then used to prepare a cDNA library from which the ligand-encoding cDNA can be isolated as disclosed above. The present invention thus provides, in addition to novel receptor polypeptides, methods for cloning polypeptide ligands for the receptors.

The tissue specificity of Zcytor7 expression suggests a role in the development of the kidney, pancreas, prostate or nervous tissues. In view of the tissue specificity observed for this receptor, agonists (including the natural ligand) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as receptor agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example, agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists may be useful in specifically promoting the growth and/or development of nervous, pancreatic or prostate-derived cells in culture. Antagonists are useful as research reagents for characterizing sites of ligand-receptor interaction. *In vivo*, receptor agonists or antagonists may find application in the treatment of renal, neural, pancreatic or prostate diseases.

Zcytor7 may also be used within diagnostic systems for the detection of circulating levels of ligand. Within a related embodiment, antibodies or other agents that specifically bind to Zcytor7 can be used to detect circulating receptor polypeptides. Elevated or depressed

levels of ligand or receptor polypeptides may be indicative of pathological conditions, including cancer.

Zcytor7 receptor polypeptides can be prepared by expressing a truncated DNA encoding the extracellular domain, for example, a polypeptide which contains residues 30 through 250 of a human Zcytor7 receptor (SEQ ID NO:2) or the corresponding region of a non-human receptor. It is preferred that the extracellular domain polypeptides be prepared in a form substantially free of transmembrane and intracellular polypeptide segments. For example, the C-terminus of the receptor polypeptide may be at residue 250 of SEQ ID NO:2 or the corresponding region of an allelic variant or a non-human receptor. To direct the export of the receptor domain from the host cell, the receptor DNA is linked to a second DNA segment encoding a secretory peptide, such as a t-PA secretory peptide. To facilitate purification of the secreted receptor domain, a C-terminal extension, such as a poly-histidine tag, substance P, Flag ™ peptide, Hopp *et al.*, *Biotechnology* 6:1204-1210 (1988), available from Eastman Kodak Co., New Haven, CT or another polypeptide or protein for which an antibody or other specific binding agent is available, can be fused to the receptor polypeptide.

In an alternative approach, a receptor extracellular domain can be expressed as a fusion with immunoglobulin heavy chain constant regions, typically an F_C fragment, which contains two constant region domains and a hinge region but lacks the variable region. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two receptor polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify the cognate ligand from solution, as an *in vitro* assay tool, to block signals *in vitro* by

specifically titrating out ligand, and as antagonists *in vivo* by administering them parenterally to bind circulating ligand and clear it from the circulation. To purify ligand, a Zcytor7-Ig chimera is added to a sample containing the ligand (e.g., cell-conditioned culture media or tissue extracts) under conditions that facilitate receptor-ligand binding (typically near-physiological temperature, pH, and ionic strength). The chimera-ligand complex is then separated by the mixture using protein A, which is immobilized on a solid support (e.g., insoluble resin beads). The ligand is then eluted using conventional chemical techniques, such as with a salt or pH gradient. In the alternative, the chimera itself can be bound to a solid support, with binding and elution carried out as above. Chimeras with high binding affinity are administered parenterally (e.g., by intramuscular, subcutaneous or intravenous injection). Circulating molecules bind ligand and are cleared from circulation by normal physiological processes. For use in assays, the chimeras are bound to a support via the F_C region and used in an ELISA format.

A preferred assay system employing a ligand-binding receptor fragment uses a commercially available biosensor instrument (BIAcore™, Pharmacia Biosensor, Piscataway, NJ), wherein the receptor fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods 145*:229-240 1991 and Cunningham and Wells, *J. Mol. Biol. 234*:554-563 (1993). A receptor fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If ligand is present in the sample, it will bind to the immobilized receptor polypeptide, causing a change in the refractive index of the medium, which is detected as a

change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity, [see, Scatchard, *Ann. NY Acad. Sci. 51*: 660-672 (1949) and calorimetric assays, Cunningham *et al.*, *Science 253*:545-548 (1991); Cunningham *et al.*, *Science 254*:821-825 (1991).

A receptor ligand-binding polypeptide can also be used for purification of ligand. The receptor polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting media will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration or pH to disrupt ligand-receptor binding.

Zcytor7 polypeptides can also be used to prepare antibodies that specifically bind to Zcytor7 epitopes, peptides or polypeptides. The Zcytor7 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. Suitable antigens would be a polypeptide comprised of a

subsequence of the Zcytor7 polypeptide against which antibodies can be produced which bind to the Zcytor7 polypeptide either in its native or denatured state. The Antibodies generated from this immune response can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, *Current Protocols in Immunology*, Cooligan, et al. (eds.), (National Institutes of Health, John Wiley and Sons, Inc., 1995); Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, (CRC Press, Inc., Boca Raton, FL, 1982).

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a Zcytor7 polypeptide or a fragment thereof. The immunogenicity of a Zcytor7 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zcytor7 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding

fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to Zcytor7 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zcytor7 protein or peptide). Genes encoding polypeptides having potential Zcytor7 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide

display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zcytor7 sequences disclosed herein to identify proteins which bind to Zcytor7. These "binding proteins" which interact with Zcytor7 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating soluble polypeptides as marker of underlying pathology or disease. These binding proteins can also act as Zcytor7 "antagonists" to block Zcytor7 binding and signal transduction *in vitro* and *in vivo*. These anti-Zcytor7 binding proteins would be useful for inhibiting ???.

Antibodies are determined to be specifically binding if: 1) they exhibit a threshold level of binding activity, and/or 2) they do not significantly cross-react with related polypeptide molecules. First, antibodies herein specifically bind if they bind to a Zcytor7 polypeptide, peptide or epitope with a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably

10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis, Scatchard, G., *Ann. NY Acad. Sci. 51*: 660-672 (1949).

Second, antibodies are determined to specifically bind if they do not significantly cross-react with related polypeptides. Antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zcytor7 but not known related polypeptides using a standard Western blot analysis (Ausubel *et al., ibid.*). Examples of known related polypeptides are orthologs, proteins from the same species that are members of a protein family (e.g. IL-16), Zcytor7 polypeptides, and non-human Zcytor7. Moreover, antibodies may be "screened against" known related polypeptides to isolate a population that specifically binds to the inventive polypeptides. For example, antibodies raised to Zcytor7 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to Zcytor7 will flow through the matrix under the proper buffer conditions. Such screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to closely related polypeptides, *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), (Cold Spring Harbor Laboratory Press, 1988); *Current Protocols in Immunology*, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, *Fundamental Immunology*, Paul (eds.), (Raven Press, 1993); Getzoff *et al.*, *Adv. in Immunol. 43*: 1-98 (1988); *Monoclonal Antibodies: Principles and Practice*, Goding, J.W. (eds.), (Academic Press Ltd., 1996); Benjamin *et al.*, *Ann. Rev. Immunol. 2*: 67-101 (1984).

A variety of assays known to those skilled in the art can be utilized to detect antibodies which specifically bind to Zcytor7 proteins or peptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), (Cold Spring Harbor Laboratory Press, 1988). Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant Zcytor7 protein or polypeptide.

Antibodies to Zcytor7 may be used for tagging cells that express Zcytor7; for isolating Zcytor7 by affinity purification; for diagnostic assays for determining circulating levels of Zcytor7 polypeptides; for detecting or quantitating soluble Zcytor7 as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block Zcytor7 $$ *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to Zcytor7 or fragments thereof may be used *in vitro* to detect denatured Zcytor7 or fragments

thereof in assays, for example, Western Blots or other assays known in the art.

Anti-idiotypic antibodies which bind to the antigenic binding site of antibodies to Zcytor7 are also considered part of the present invention. The antigenic binding region of the anti-idiotypic antibody thus will mimic the ligand binding region of Zcytor7. An anti-idiotypic antibody thus could be used to screen for possible ligands of the Zcytor7 receptor. Thus neutralizing antibodies to Zcytor7 can be used to produce anti-idiotypic antibodies by methods well known in the art as is described in, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, (CRC Press, Inc., Boca Raton, FL, 1982).

The present invention also provides reagents which will find use in diagnostic applications. For example, the Zcytor7 gene, a probe comprising Zcytor7 DNA or RNA or a subsequence thereof can be used to determine if the Zcytor7 gene is present on chromosome 6 or if a mutation has occurred. Detectable chromosomal aberrations at the Zcytor7 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., *ibid.*; Ausubel *et. al.*, *ibid.*; Marian, *Chest* *108*:255-65, 1995).

Transgenic mice, engineered to express the Zcytor7 gene, and mice that exhibit a complete absence of Zcytor7 gene function, referred to as "knockout mice", Snouwaert *et al.*, *Science* 257:1083 (1992), may also be generated, Lowell *et al.*, *Nature* 366:740-42 (1993). These mice may be employed to study the Zcytor7 gene and the protein encoded thereby in an *in vivo* system.

CHROMOSOMAL LOCALIZATION:

Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes, Cox *et al.*, *Science* 250:245-50, (1990). Partial or full knowledge of a gene's sequence allows one to design PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR-based chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other nonpolymorphic and polymorphic markers within a region of interest. This includes establishing directly proportional physical distances between newly discovered genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

Sequence tagged sites (STSs) can also be used independently for chromosomal localization. An STS is a DNA sequence that is unique in the human genome and can be used as a reference point for a particular chromosome or region of a chromosome. An STS is defined by a pair of oligonucleotide primers that are used in a polymerase chain reaction to specifically detect this site in the presence of all other genomic sequences. Since STSs are based solely on DNA sequence they can be completely described within an electronic database, for example, Database of Sequence Tagged Sites (dbSTS), GenBank, (National Center for Biological Information, National Institutes of Health, Bethesda, MD http://www.ncbi.nlm.nih.gov), and can be searched with a gene sequence of interest for the mapping data contained within these short genomic landmark STS sequences.

Zcytor-7 maps 795.76 cR from the top of the human chromosome 6 linkage group on the WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was CHLC.GATA32B03 and its nearest distal maker was SGC32063. The use of surrounding markers also helped position zcytor-7 in the 6q22-q23 region on the CHLC chromosome 6 version v8c7 integrated marker map. The locus where Zcytor7 maps onto chromosome 6 is a common breakpoint area in ALL(acute lymphoblastic leukemia) and NHL(non-Hodgkin lymphoma) as well as in AML(acute myelogenous leukemia) and CML(chronic myeloid leukemia). It is interesting to note that the MYB (avian myeloblastosis viral oncogene homolog) gene, which encodes proteins critical for hematopoetic cell proliferation and development, appears to be less than 800 kB from Zcytor7. The 6q- deletion breakpoints occur slightly distal to the

MYB gene and although the neoplasms show high levels of MYB mRNA, the gene itself appears to be intact.

Thus Zcytor7 could be used to generate a probe that could allow detection of an aberration of the Zctyor7 gene in the 6q chromosome which may indicate the presence of a cancerous cell such as leukemic cells which may still be present in after chemical or radiation therapy. If the Zcytor7 gene is deleted by the chromosomal abnormality, only one copy can be used to determine whether one or two copies of the gene are present per nucleus, thus indicating the percentage cancerous cells might be present relative to normal cells. For further discussions on developing polynucleotide probes and hybridization see *Current Protocols in Molecular Biology* Ausubel, F. *et al.* Eds. (John Wiley & Sons Inc. 1991).

Pharmaceutical Compositions

Pharmaceutical compositions can be formulated which contain the soluble receptor, antibody or anti-idiotypic antibodies of the present invention. Generally included in such protein therapeutic compositions are buffers; surface adsorption inhibitors such as surfactants and polyols; and isotonic amounts of a physiologically acceptable salt. The composition may be formulated as an aqueous solution or a lyophilized powder. The latter is reconstituted prior to use with a pharmaceutically acceptable diluent such as sterile water for injection.

Examples of buffers which can be used for the above-described pharmaceutical compositions include low ionic strength, physiologically acceptable buffers that are effective within the pH range of 5.0 - 7.0. Such buffers include phosphate, acetate, citrate, succinate and histidine buffers.

Examples of surface adsorption inhibitors which can be used in the above-described pharmaceutical compositions include non-ionic surfactants and polyols. Non-ionic surfactants include polyoxyethylene sorbitan fatty acid esters, such as polysorbate 20 (polyoxyethylene sorbitan monolaurate), and the like. Other non-ionic surfactants useful in this regard include polyethylene oxides; sorbitan esters; polyoxyethylene alkyl ethers; and glycerides of fatty acid, including glyceryl monooleate and glyceryl monostearate. Polyols which can be used include polyethylene glycol, *e.g.* PEG 3350, mannitol, xylitol, sorbitol, inositol, and glycerol. In general, the surface adsorption inhibitor will be included within the composition at a concentration from 0.001% to 5%.

Physiologically acceptable salts are generally included in a protein therapeutic composition generally in an amount isotonic to human blood. Preferred salts in this regard include chloride salts such as NaCl, KCl, $CaCl_2$ and $MgCl_2$.

Albumin may also be included in the above-described pharmaceutical compositions. Human serum albumin is preferred for inclusion in pharmaceutical compositions intended for human use. Albumin is useful as an excipient in lyophilized compositions and acts as a stabilizer when included at a concentration of 0.1 - 1.0%. Albumin may useful as a surface adsorption inhibitor.

One or more preservatives may also be included in the pharmaceutical compositions of the present invention. Common preservative include methylparaben, propylparaben, benzyl alcohol, m-cresol, ethylmercurithiosalycilate, phenol, thimerosol and the like. Methods of formulation of pharmaceutical compositions are well known in the art and are disclosed, for example, in *Remington's Pharmaceutical*

Sciences, Gennaro, ed., (Mack Publishing Co., Easton, PA, 1996)

Dosages

Therapeutic doses of the protein compositions of the present invention will generally be in the range of 0.1 to 100 µg/kg of patient per day with the exact dose determined by the clinician.

The invention is further illustrated by the following non-limiting examples.

Example 1.

Cloning of Zcytor7

Expressed sequence tag SEQ ID NO:3 was identified. A cDNA clone was discovered which contained a partial sequence. The cDNA sequence was determined to be 1231 bp in length, but was not a full length sequence.

A human testis cDNA template was made using a MARATHON™ cDNA Amplification Kit (Clontech Laboratories, Inc., Palo Alto, CA) according to the supplier's instructions. A 5' RACE reaction was used to obtain a full-length cDNA. The RACE reaction was carried out in two reactions employing two sets of primers. Reaction I (outer nest), using primers SEQ ID NO:4 and AP-1 (SEQ ID NO: 5) (Clontech Laboratories) was run for 35 cycles at 98°C for 20 seconds, 45°C for 20 seconds; 68° for 4 minutes and a final extension time of 10 minutes at 68°C. One µl of a 1:100 dilution of the reaction product was used as a template in reaction II (inner nest). Primers were ZC11,108 (SEQ ID NO:6) and AP-2 (SEQ ID NO:7) (Clontech Laboratories). The reaction was run at 98°C for 30 seconds, and 30 cycles each cycle being comprised of 98°C for 28

seconds; 43°C for 20 seconds; and 68°C for 3.5 minutes with a final extension at 68°C for 10 minutes.

The product of the inner nest RACE reaction was subcloned using a PCR-SCRIPT™ kit (Stratagene Cloning Systems, La Jolla, CA) to prepare the plasmid pSLR7-1. Sequence analysis of this plasmid indicated that the 5'RACE-generated sequence extended the sequence of pSL7139 by 555 bp.

Full-length cDNA was obtained by screening a λZAP® II human testis cDNA library using a probe that was generated by PCR primers ZC11,526 (SEQ ID NO:9) and ZC11,108 (SEQ ID NO:6) and pSLR7-1 as template and then re-amplified. The resulting probe was purified through recovery from low-melt agarose gel electrophoresis and was labeled with ^{32}P-α-dCTP using a MEGAPRIME™ labeling kit (Amersham Corp., Arlington, Heights, IL). The labeled probe was purified on a push column (NUCTRAP® probe purification column; Stratagene Cloning Systems).

The first strand cDNA reaction contained 15 µl of human testis twice poly d(T)-selected poly $(A)^+$ mRNA (Clontech Laboratories) at a concentration of 1.0 µg/µl, and 3 µl of 20 pmole/µl first strand primer ZC6091 (SEQ ID NO:8) containing an *Xho* I restriction site. The mixture was heated at 70°C for 4 minutes and cooled by chilling on ice. First stand cDNA synthesis was initiated by the addition of 12 µl of first strand buffer (5x SUPERSCRIPT™ buffer; Life Technologies, Gaithersburgh, MD), 6 µl of 100 mM dithiothreitol, 3 µl of deoxynucleotide triphosphate solution containing 10 mM each of dTTP, dATP, dGTP, and 5-methyl-dCTP (Pharmacia LKB Biotechnology, Piscataway, NJ) to the RNA-primer mixture. The reaction mixture was incubated at 37°C for 2 minutes, followed by the addition of 15 µl of 200 U/µl Rnase H^- reverse transcriptase

(SUPERSCRIPT II® ; Life Technologies). The efficiency of the first strand synthesis was analyzed in a parallel reaction by the addition of 5 μCi of ^{32}P-αdCTP to 5 μl aliquot from one of the reaction mixtures to label the reaction for analysis. The reactions were incubated at 37°C for 10 minutes, 45°C for 1 hour, then incubated at 50°C for 10 minutes. Unincorporated ^{32}P-αdCTP in the labeled reaction and the unincorporated nucleotides and primers in the unlabeled first strand reactions were removed by chromatography on a 400 pore size gel filtration column (Clontech Laboratories). The length of labeled first strand cDNA was determined by agarose gel electrophoresis.

The second strand reaction contained 120 μl of the unlabeled first strand cDNA, 36 μl of 5x polymerase I buffer (125 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM $MgCl_2$,50mM $(NH_4)_2SO_4$)), 2.4 μl of 100 mM dithiothreitol, 3.6 μl of a solution containing 10 mM of each deoxynucleotide triphosphate, 6 μl of 5 mM β-NAD, 3.6 μl of 3 U/μl *E. coli* DNA ligase (New England Biolabs),9 μl of 10 U/μl *E. coli* DNA polymerase I (New England Biolabs), and 1.8 μl of 2 U/μl RNase H (life Technologies). A 10 μl aliquot from one of the second strand synthesis reactions was labeled by the addition of 10 μCi ^{32}P-αdCTP to monitor the efficiency of second strand synthesis. The reactions were incubated at 16°C for two hours, followed by the addition of 15 μl T4 DNA polymerase (10 U/μl, Boerhinger Mannheim, Indianapolis, IN) and incubated for an additional 5 minutes at 16°C. Unincorporated ^{32}P-αdCTP in the labeled reaction was removed by chromatography through a 400 pore size gel filtration (Clontech Laboratories) before analysis by agarose gel electrophoresis. The unlabeled second strand reaction was terminated by the addition of 20 μl 0.5 M EDTA and extraction with phenol/chloroform and chloroform followed by ethanol precipitation in the presence of 2.5 M ammonium acetate and 4 μg of glycogen

carrier. The yield of cDNA was estimated to be approximately 3 µg from starting mRNA template of 15 µg.

Eco RI adapters were ligated onto the 5'ends of the cDNA described above to enable cloning into an expression vector. A 10 µl aliquot of cDNA (approximately 1.5 µg) and 5 µl of 65 pmole/µl of *Eco* RI adapter (Pharmacia LKB Biotechnology Inc.) were mixed with 2 µl 10x ligase buffer (660 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$), 2 µl of 10 mM ATP and 1 µl of 15 U/µl T4 DNA ligase (Promega Corp., Madison, WI). The reaction was incubated 2 hours at 5°C, two hours at 7.5°C, 2 hours at 10°C, and 10 hours at 12.5°C. The reaction was terminated by incubation at 70°C for 20 minutes.

To facilitate the directional cloning of the cDNA into an expression vector, the cDNA was digested with *Xho* I, resulting in a cDNA having a 5' *Eco* RI cohesive end and a 3' *Xho* cohesive end. The *Xho* I restriction site at the 3'end of the cDNA had been previously introduced using the ZC6091 primer (SEQ ID NO: 8). Restriction enzyme digestion was carried out in a reaction mixture containing 20 µl of cDNA as described above, 10 µl of 10x H Buffer *Xho* I (Boehringer Mannheim), 69 µl H_2O,and 1.0 µl of 40 U/µl *Xho* I (Boehringer Mannheim). Digestion was carried out at 37°C for 40 minutes. The reaction was terminated by incubation at 70°C for 10 minutes and chromatography through a 400 pore size gel filtration column (Clontech Laboratories).

The cDNA was ethanol precipitated, washed with 70% ethanol, air dried and resuspended in 14 µl water, 2 µl of ligase buffer (Promega Corp., Madison, WI), 2 µl T4 polynucleotide kinase (10 U/µl, Life Technologies). Following incubation at 37°C for 30 minutes, the cDNA was heated to 65°C for 5 minutes, cooled on ice, and electrophoresed on a 0.8% low melt agarose gel. The

contaminating adapters and cDNA below 0.6 kb in length were excised from the gel. The electrodes were reversed, and the cDNA was electrophoresed until concentrated near the lane origin. The area of the gel containing the concentrated cDNA was excised and placed in a microfuge tube, and the approximate volume of the gel slice was determined. An aliquot of water approximately three times the volume of the gel slice (300 µl) and 35 µl 10x β-agarose I buffer (New England Biolabs) were added to the tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the sample to 45°C, 3 µl of 1 U/µl β-agarose I (New England Biolabs) was added, and the mixture was incubated for 60 minutes at 45°C to digest the agarose. After incubation, 40 µl of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose. The cDNA was ethanol precipitated, washed in 70% ethanol, air-dried and resuspended in 10 µl water.

The resulting cDNA was cloned into the lambda phage vector λZap® II (Stratagene Cloning Systems) that was predigested with *Eco* RI and *Xho* I and dephosphorylated. Ligation of the cDNA to the λZap® II vector was carried out in a reaction mixture containing 1.0 µl of prepared vector, 1.0 µl of human testis cDNA, 1.0 µl 10X Ligase Buffer (Promega Corp.), 1.0 µl of 10 mM ATP, 5 µl water, and 1.0 µl of T4 DNA Ligase at 15 units/ml (Promega Corp.). The ligation mixture was incubated at 5°-15°C overnight in a temperature gradient. After incubation, the ligation mixture was packaged into phage using an *in vitro* packaging extract (Gigapack® III Gold packaging extract; Stratagene Cloning Systems), and the resulting library was titered according to the manufacturer's specifications.

The human testis λZAP® II library was used to infect *E. coli* host cells (XL1-Blue MRF' strain (Stratagene Cloning Systems), and 1.5 x 10^6 plaque forming units (pfu) were plated onto 150-mm NZY plates at a density of about 50,000 pfu/plate. The inoculated plates were incubated overnight at 37°C. Filter plaque lifts were made using nylon membranes (Hybond™-N; Amersham Corp., Arlington Heights, IL), according to the procedures provided by the manufacturer. The filters were processed by denaturation in solution containing 1.5 M NaCl and 0.5 M NaOH for 6 minutes at room temperature. The filters were blotted briefly on filter paper to remove excess denaturation solution, followed by neutralization for 6 minutes in 1 M Tris-HCl, pH 7.5, and 1.5 M NaCl. Phage DNA was fixed onto the filters with 1,200 µJoules of UV energy in a UV Crosslinker (Stratalinker®; Stratagene Cloning Systems). After fixing, the filters were first pre-washed in an aqueous solution containing .25X standard sodium citrate (SSC),.25% sodium dodecyl sulfate (SDS) and 1mM EDTA to remove cellular debris and then prehybridized in hybridization solution (5X SSC, 5X Denhardt's solution, 0.2% SDS and 1 mM EDTA). Heat-denatured, sheared salmon sperm DNA at a final concentration of 100 µg/ml was added. The filters were prehybridized at 65°C overnight.

A probe was prepared as a PCR product by using oligonucleotide primers designed to amplify the human Zcytor7 coding region. A PCR reaction mixture was prepared containing 2 µl of ZC11526 (SEQ ID NO:9) 2 µl of ZC11,108 (SEQ ID NO:6), 1 µl of an overnight bacterial culture of pSLR7-1, 1 µl of 10 mM dNTP, 10 µl of 10X KlenTaq buffer (Clontech Laboratories), 82 µl water, and 2 µl KlenTaq DNA polymerase (Clontech laboratories). The PCR reaction was run as follows: 94°C for 1 minute; 30 cycles of 95°C for 20 seconds, 43°C for 20 seconds, 68°C for 1 minute; then held

at 68°C for 10 minutes. The PCR product was re-amplified and gel purified on a .8% low melt agarose gel.

Fifty nanograms PCR product was radiolabeled with ^{32}P-α-dCTP by random priming using the MEGAPRIME® DNA Labeling System (Amersham), according to the manufacturer's specifications. The prehybridization solution was replaced with fresh hybridization solution containing 1.4 x 10^6cpm/ml labeled probe and allowed to hybridize for 64 hours at 60°C. After hybridization, the hybridization solution was removed and the filters were rinsed in a wash solution containing .25X SSC, 0.25% SDS and 1 mM EDTA at 65°C. The filters were placed on autoradiograph film and exposed at -70°C with intensifying screens for 72 hours.

Examination of the autoradiographs revealed multiple regions that hybridized with labeled probe. Agar plugs were picked from 12 regions for purification. Each agar plug was soaked 2 hours in 0.5 ml of SM solution containing 25 ml 4M NaCl, 10 ml 1M $MgSO_4$, 25 ml 2M Tris HCl, 5 ml 2% gelatin and 935 ml H_2O and 10% (v/v) chloroform (Sambrook *et al. Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). After incubation, the phages from each plug were diluted 1:1000 in SM. Aliquots of 50 µl were plated on 100 mm plates containing 300 µl of *E. coli* XL-1 Blue MRF' cells. The plates were incubated overnight at 37°C, and filter lifts were prepared, prehybridized overnight, hybridized overnight with a hybridization solution containing 1.1 x 10^6 cpm/ml labeled probe, washed and autoradiographed. Examination of the resulting autoradiographs revealed 10 positive signals. The positive plaques were subjected to an additional round of purification.

The plasmids were excised using an ExASSIST/SOLR® system (Stratagene Cloning Systems), according to the manufacturer's specifications. These plasmid inserts were amplified by PCR for size determination. A clone, designated pSLR7-2 was sequenced and determined to have an insert of 3,532 bp in size.

Example 2
Northern Blot Analysis

A 970 bp fragment of the Zcytor7 cDNA containing nucleotides 822 - 1791 was random primer labeled using a MULTIPRIME™ kit (Amersham Corp.). Labeled cDNA was purified from free counts using a push column (Stratagene Cloning Systems). A human RNA master dot blot (Clontech Laboratories) for three hours at 65°C, then hybridized with 10^6cpm/ml of labeled cDNA probe. The expression pattern for this blot, which contained RNA samples which had been normalized to the mRNA expression levels of eight different housekeeping genes, was highest in kidney, followed by spinal cord, prostate, and cerebellum.

Example 3
Expression of Human Zcytor7 mRNA in Human Tissues

Poly(A)$^+$ RNAs isolated from adrenal cortex, adrenal medulla, brain, colon, heart, kidney, liver, lung, ovary, pancreas, prostate, placenta, peripheral blood leukocytes, stomach, spleen, skeletal muscle, small intestine, testis, thymus, thyroid, fetal brain, fetal lung, fetal liver and fetal kidney were hybridized under high stringency conditions with a radiolabeled DNA probe containing nucleotides 822-1791 of (SEQ ID NO:1). Membranes were purchased from Clontech. The membrane were washed with 0.1X SSC, 0.1% SDS at 50°C and autoradiographed for 24 hours. The mRNA levels were highest in adrenal cortex,

pancreas and prostate with lower levels in testis, stomach, adrenal medulla and thyroid.

Example 4

Chromosomal Assignment and Placement of Zcytor-7

Zcytor-7 was mapped to chromosome 6 using the commercially available version of the Whitehead Institute/MIT Center for Genome Research's "GeneBridge 4 Radiation Hybrid Panel" (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel contains PCRable DNAs from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23 recipient). A publicly available WWW server (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) allows mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome (the "WICGR" radiation hybrid map) which was constructed with the GeneBridge 4 Radiation Hybrid Panel.

For the mapping of zcytor-7 with the "GeneBridge 4 RH Panel", 25 µl reactions were set up in a PCRable 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a "RoboCycler Gradient 96" thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2.5 µl 50X "Advantage KlenTaq Polymerase Mix" (CLONTECH Laboratories, Inc., Palo Alto, CA), 2 µl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1.25 µl sense primer, SEQ ID NO: 11, 1.25 µl antisense primerSEQ ID NO: 12, 2.5 µl "RediLoad" (Research Genetics, Inc., Huntsville, AL), 0.5 µl "Advantage KlenTaq Polymerase Mix" (Clontech Laboratories, Inc.), 25 ng of DNA from an individual hybrid clone or control and water is added to bring up the total volume to 25 µl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as

follows: an initial 1 cycle 4 minute denaturation at 94°C, 35 cycles of a 1 minute denaturation at 94°C, 1.5 minute annealing at 63°C and 1.5 minute extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 3% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME).

The results showed that zcytor-7 maps 795.76 cR from the top of the human chromosome 6 linkage group on the WICGR radiation hybrid map. Relative to the centromere, its nearest proximal marker was CHLC.GATA32B03 and its nearest distal maker was SGC32063. The use of surrounding markers also helped position zcytor-7 in the 6q22-q23 region on the CHLC chromosome 6 version v8c7 integrated marker map (The Cooperative Human Linkage Center, WWW server: http:// www.chlc.org/ChlcIntegratedMaps.html) and to 6q22.33-q23.1 on the integrated LDB chromosome 6 map (The Genetic Location Database, University of Southhampton, WWW server:http://cedar.genetics.soton.ac.uk/public_html/).

This is a common breakpoint area in ALL(acute lymphoblastic leukemia) and NHL(non-Hodgkin lymphoma) as well as in AML(acute myelogenous leukemia) and CML(chronic myeloid leukemia). It is interesting to note that the MYB (avian myeloblastosis viral oncogene homolog) gene, which encodes proteins critical for hematopoetic cell proliferation and development, appears to be less than 800 kB from Zcytor7. The 6q- deletion breakpoints occur slightly distal to the MYB gene and although the neoplasms show high levels of MYB mRNA, the gene itself appears to be intact.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: ZymoGenetic, Inc.
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(ii) TITLE OF THE INVENTION: CYTOKINE RECEPTOR

(iii) NUMBER OF SEQUENCES: 13

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(F) ZIP: 98102

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/803,305
(B) FILING DATE: 20-FEB-1997

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3516 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 237...1895
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TCCAGCTGGG TAGCCGGGGG AGCGCGCGTG GGGGCTCCGC GAGTCGCTCG CCCTTGGTTT    60
CTGGGGAAGC CTGGGGGACG CGGCTGTGGC GGAGGCGCCC TGGGACTCAG GTCGCCTGGA   120
GCGTGGCACG CAGAGCCCCA GGCGCGGAGC TGAGGCCGCG CGGCCGCGCT TGGCCCCAGC   180
GGGCGTGGGA CTGAGCAGTC TGCTGCCCCC CGACATGTGA CCCAGCCCCG CCGCCC ATG   239
                                                                 Met
                                                                  1

CGG GCT CCC GGC CGC CCG GCC CTG CGG CCG CTG CCG CTG CCG CCG CTG     287
Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Leu Pro Leu Pro Pro Leu
            5                  10                  15

CTG CTG TTG CTC CTG GCG GCG CCT TGG GGA CGG GCA GTT CCC TGT GTC     335
Leu Leu Leu Leu Leu Ala Ala Pro Trp Gly Arg Ala Val Pro Cys Val
        20                  25                  30

TCT GGT GGT TTG CCT AAA CCT GCA AAC ATC ACC TTC TTA TCC ATC AAC     383
Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe Leu Ser Ile Asn
    35                  40                  45
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ATG AAG AAT GTC CTA CAA TGG ACT CCA CCA GAG GGT CTT CAA GGA GTT      431
Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu Gly Leu Gln Gly Val
50                  55                  60                  65

AAA GTT ACT TAC ACT GTG CAG TAT TTC ATA TAT GGG CAA AAG AAA TGG      479
Lys Val Thr Tyr Thr Val Gln Tyr Phe Ile Tyr Gly Gln Lys Lys Trp
                70                  75                  80

CTG AAT AAA TCA GAA TGC AGA AAT ATC AAT AGA ACC TAC TGT GAT CTT      527
Leu Asn Lys Ser Glu Cys Arg Asn Ile Asn Arg Thr Tyr Cys Asp Leu
            85                  90                  95

TCT GCT GAA ACT TCT GAC TAC GAA CAC CAG TAT TAT GCC AAA GTT AAG      575
Ser Ala Glu Thr Ser Asp Tyr Glu His Gln Tyr Tyr Ala Lys Val Lys
        100                 105                 110

GCC ATT TGG GGA ACA AAG TGT TCC AAA TGG GCT GAA AGT GGA CGG TTC      623
Ala Ile Trp Gly Thr Lys Cys Ser Lys Trp Ala Glu Ser Gly Arg Phe
    115                 120                 125

TAT CCT TTT TTA GAA ACA CAA ATT GGC CCA CCA GAG GTG GCA CTG ACT      671
Tyr Pro Phe Leu Glu Thr Gln Ile Gly Pro Pro Glu Val Ala Leu Thr
130                 135                 140                 145

ACA GAT GAG AAG TCC ATT TCT GTT GTC CTG ACA GCT CCA GAG AAG TGG      719
Thr Asp Glu Lys Ser Ile Ser Val Val Leu Thr Ala Pro Glu Lys Trp
                150                 155                 160

AAG AGA AAT CCA GAA GAC CTT CCT GTT TCC ATG CAA CAA ATA TAC TCC      767
Lys Arg Asn Pro Glu Asp Leu Pro Val Ser Met Gln Gln Ile Tyr Ser
            165                 170                 175

AAT CTG AAG TAT AAC GTG TCT GTG TTG AAT ACT AAA TCA AAC AGA ACG      815
Asn Leu Lys Tyr Asn Val Ser Val Leu Asn Thr Lys Ser Asn Arg Thr
        180                 185                 190

TGG TCC CAG TGT GTG ACC AAC CAC ACG CTG GTG CTC ACC TGG CTG GAG      863
Trp Ser Gln Cys Val Thr Asn His Thr Leu Val Leu Thr Trp Leu Glu
    195                 200                 205

CCG AAC ACT CTT TAC TGC GTA CAC GTG GAG TCC TTC GTC CCA GGG CCC      911
Pro Asn Thr Leu Tyr Cys Val His Val Glu Ser Phe Val Pro Gly Pro
210                 215                 220                 225
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CCT CGC CGT GCT CAG CCT TCT GAG AAG CAG TGT GCC AGG ACT TTG AAA      959
Pro Arg Arg Ala Gln Pro Ser Glu Lys Gln Cys Ala Arg Thr Leu Lys
                230                 235                 240

GAT CAA TCA TCA GAG TTC AAG GCT AAA ATC ATC TTC TGG TAT GTT TTG     1007
Asp Gln Ser Ser Glu Phe Lys Ala Lys Ile Ile Phe Trp Tyr Val Leu
            245                 250                 255

CCC ATA TCT ATT ACC GTG TTT CTT TTT TCT GTG ATG GGC TAT TCC ATC     1055
Pro Ile Ser Ile Thr Val Phe Leu Phe Ser Val Met Gly Tyr Ser Ile
        260                 265                 270

TAC CGA TAT ATC CAC GTT GGC AAA GAG AAA CAC CCA GCA AAT TTG ATT     1103
Tyr Arg Tyr Ile His Val Gly Lys Glu Lys His Pro Ala Asn Leu Ile
    275                 280                 285

TTG ATT TAT GGA AAT GAA TTT GAC AAA AGA TTC TTT GTG CCT GCT GAA     1151
Leu Ile Tyr Gly Asn Glu Phe Asp Lys Arg Phe Phe Val Pro Ala Glu
290                 295                 300                 305

AAA ATC GTG ATT AAC TTT ATC ACC CTC AAT ATC TCG GAT GAT TCT AAA     1199
Lys Ile Val Ile Asn Phe Ile Thr Leu Asn Ile Ser Asp Asp Ser Lys
                310                 315                 320

ATT TCT CAT CAG GAT ATG AGT TTA CTG GGA AAA AGC AGT GAT GTA TCC     1247
Ile Ser His Gln Asp Met Ser Leu Leu Gly Lys Ser Ser Asp Val Ser
            325                 330                 335

AGC CTT AAT GAT CCT CAG CCC AGC GGG AAC CTG AGG CCC CCT CAG GAG     1295
Ser Leu Asn Asp Pro Gln Pro Ser Gly Asn Leu Arg Pro Pro Gln Glu
        340                 345                 350

GAA GAG GAG GTG AAA CAT TTA GGG TAT GCT TCG CAT TTG ATG GAA ATT     1343
Glu Glu Glu Val Lys His Leu Gly Tyr Ala Ser His Leu Met Glu Ile
    355                 360                 365

TTT TGT GAC TCT GAA GAA AAC ACG GAA GGT ACT TCT TTC ACC CAG CAA     1391
Phe Cys Asp Ser Glu Glu Asn Thr Glu Gly Thr Ser Phe Thr Gln Gln
370                 375                 380                 385

GAG TCC CTC AGC AGA ACA ATA CCC CCG GAT AAA ACA GTC ATT GAA TAT     1439
Glu Ser Leu Ser Arg Thr Ile Pro Pro Asp Lys Thr Val Ile Glu Tyr
                390                 395                 400
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GAA TAT GAT GTC AGA ACC ACT GAC ATT TGT GCG GGG CCT GAA GAG CAG    1487
Glu Tyr Asp Val Arg Thr Thr Asp Ile Cys Ala Gly Pro Glu Glu Gln
            405                 410                 415

GAG CTC AGT TTG CAG GAG GAG GTG TCC ACA CAA GGA ACA TTA TTG GAG    1535
Glu Leu Ser Leu Gln Glu Glu Val Ser Thr Gln Gly Thr Leu Leu Glu
        420                 425                 430

TCG CAG GCA GCG TTG GCA GTC TTG GGC CCG CAA ACG TTA CAG TAC TCA    1583
Ser Gln Ala Ala Leu Ala Val Leu Gly Pro Gln Thr Leu Gln Tyr Ser
    435                 440                 445

TAC ACC CCT CAG CTC CAA GAC TTA GAC CCC CTG GCG CAG GAG CAC ACA    1631
Tyr Thr Pro Gln Leu Gln Asp Leu Asp Pro Leu Ala Gln Glu His Thr
450                 455                 460                 465

GAC TCG GAG GAG GGG CCG GAG GAA GAG CCA TCG ACG ACC CTG GTC GAC    1679
Asp Ser Glu Glu Gly Pro Glu Glu Glu Pro Ser Thr Thr Leu Val Asp
                470                 475                 480

TGG GAT CCC CAA ACT GGC AGG CTG TGT ATT CCT TCG CTG TCC AGC TTC    1727
Trp Asp Pro Gln Thr Gly Arg Leu Cys Ile Pro Ser Leu Ser Ser Phe
            485                 490                 495

GAC CAG GAT TCA GAG GGC TGC GAG CCT TCT GAG GGG GAT GGG CTC GGA    1775
Asp Gln Asp Ser Glu Gly Cys Glu Pro Ser Glu Gly Asp Gly Leu Gly
        500                 505                 510

GAG GAG GGT CTT CTA TCT AGA CTC TAT GAG GAG CCG GCT CCA GAC AGG    1823
Glu Glu Gly Leu Leu Ser Arg Leu Tyr Glu Glu Pro Ala Pro Asp Arg
    515                 520                 525

CCA CCA GGA GAA AAT GAA ACC TAT CTC ATG CAA TTC ATG GAG GAA TGG    1871
Pro Pro Gly Glu Asn Glu Thr Tyr Leu Met Gln Phe Met Glu Glu Trp
530                 535                 540                 545

GGG TTA TAT GTG CAG ATG GAA AAC TGATGCCAAC ACTTCCTTTT GCCTTTTGTT    1925
Gly Leu Tyr Val Gln Met Glu Asn
                550

TCCTGTGCAA ACAAGTGAGT CACCCCTTTG ATCCCAGCCA TAAAGTACCT GGGATGAAAG    1985
AAGTTTTTTC CAGTTTGTCA GTGTCTGTGA GAATTACTTA TTTCTTTTCT CTATTCTCAT    2045
AGCACGTGTG TGATTGGTTC ATGCATGTAG GTCTCTTAAC AATGATGGTG GGCCTCTGGA    2105
GTCCAGGGGC TGGCCGGTTG TTCTATGCAG AGAAAGCAGT CAATAAATGT TTGCCAGACT    2165
GGGTGCAGAA TTTATTCAGG TGGGTGTACT CTGGCCTCTT GGTTCATTAT TTTCAAACAA    2225
```

GCACACTTGT ACAATTATTT TCTGGGTACT TCCCATATGC ACATAGCACT GTAAAAAATA 2285
TTTCCCAAAG ATCACTCATT TTATAAATAC CACTTTTTCA GAATTGGGTT TATTGCGAGC 2345
AGGAGGAGAT ACTTAAAACA TGCACATATA CCAGGTTGGT GGTAAGTTGG TCACATGTGA 2405
AAACCTCAAC TATTTAATCA TCATGATTCA TATTTTGAGT GAATACATCA GGCACAGACC 2465
TTCATGATAT CACACACTCT TGGCTACTTT AAGAGGCCAT CTTTAATACT TTATGAGTAG 2525
TTCTGGAGTG TAAACATAAA CGAGTATTCT TTTGTAGTCA GAAAAGTGTC CTCTCAATAA 2585
TTTAGTAGGG GCTTATTGTC TCTCAAAACT AACCTAAAAG AAAATGACAC ATTTTATAAT 2645
AGAATATTAC ATTTATTTCT GGAAGTGTGT TTTCAAAAAG ATATTTACAT AGTCTGTAAA 2705
CTAGAAAGTG TTAGGTAAAG CTCTAGGTTA CTGTGTTACT ATTATAATAT TAAACATTCG 2765
AATAGGCAGT CGTTCAAAGA CTCTTTGGAA TATCTATGAA TGAATATCCT CTATTCTTAT 2825
AATATTAAAA CCCATAAGTA AATATAGGAC ATACAAGAGA AATGAGTTAA ATGACTATGT 2885
AAGGGAGAGT TTATTAAAAT TTGATGAAAT TTACTGTAGG AACTAAACTA TGCCATAAAA 2945
CAATAGCTTT CTAGTTCATT TCCAGTAACT GTTCCCATCT CCTTTACCAC TTGTTAAGAA 3005
AATTAAATTC TTCAGTCACG CTGCTTTAAA ATGGGACAAA ATCTATTAAG TTGAACCATA 3065
TATAATTGTG GATATTTGGC TGTTTTTAAT CTGACAAGCA GTAACTTCAT ATGGTTTGCC 3125
TTAATATATA TTTGTTTTAG TCATGAACTC ATAATCCATT GATGCTCTTT CATGAGAAGA 3185
GATATGACCC ATATTTCCTT ATTGATATTA TTGGTACAGG CAGACAACCC TGGTAGGAGA 3245
GATGGATTCT GGGGTCATGA CCTTTCGTGA TTATCCGCAA ATGCAAACAG TTTCAGATCT 3305
AATGGTTTAA TTTAGGGAGT AATTATATTA ATCAGAGTGT TCTGTTATTC TCAATCTTTA 3365
TAGAAACGAT TCTGCTGGTT TTGAAGAACA GATGTATTAC ACTAACTGTA AAAGTAGTTC 3425
AAGAGTGAGA AAGAATAAAT TGTTATTAAG AGCAAAAGAA AAATAAAGTG ATTGATGATA 3485
AAAAAAAAAA AAAAAAAGCG GCCGCCTCGA G 3516

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 553 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Leu Pro Leu Pro Pro
 1               5                  10                  15
Leu Leu Leu Leu Leu Leu Ala Ala Pro Trp Gly Arg Ala Val Pro Cys
            20                  25                  30
Val Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe Leu Ser Ile
        35                  40                  45
Asn Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu Gly Leu Gln Gly
    50                  55                  60
```

Val Lys Val Thr Tyr Thr Val Gln Tyr Phe Ile Tyr Gly Gln Lys Lys
65 70 75 80
Trp Leu Asn Lys Ser Glu Cys Arg Asn Ile Asn Arg Thr Tyr Cys Asp
85 90 95
Leu Ser Ala Glu Thr Ser Asp Tyr Glu His Gln Tyr Tyr Ala Lys Val
100 105 110
Lys Ala Ile Trp Gly Thr Lys Cys Ser Lys Trp Ala Glu Ser Gly Arg
115 120 125
Phe Tyr Pro Phe Leu Glu Thr Gln Ile Gly Pro Pro Glu Val Ala Leu
130 135 140
Thr Thr Asp Glu Lys Ser Ile Ser Val Val Leu Thr Ala Pro Glu Lys
145 150 155 160
Trp Lys Arg Asn Pro Glu Asp Leu Pro Val Ser Met Gln Gln Ile Tyr
165 170 175
Ser Asn Leu Lys Tyr Asn Val Ser Val Leu Asn Thr Lys Ser Asn Arg
180 185 190
Thr Trp Ser Gln Cys Val Thr Asn His Thr Leu Val Leu Thr Trp Leu
195 200 205
Glu Pro Asn Thr Leu Tyr Cys Val His Val Glu Ser Phe Val Pro Gly
210 215 220
Pro Pro Arg Arg Ala Gln Pro Ser Glu Lys Gln Cys Ala Arg Thr Leu
225 230 235 240
Lys Asp Gln Ser Ser Glu Phe Lys Ala Lys Ile Ile Phe Trp Tyr Val
245 250 255
Leu Pro Ile Ser Ile Thr Val Phe Leu Phe Ser Val Met Gly Tyr Ser
260 265 270
Ile Tyr Arg Tyr Ile His Val Gly Lys Glu Lys His Pro Ala Asn Leu
275 280 285
Ile Leu Ile Tyr Gly Asn Glu Phe Asp Lys Arg Phe Phe Val Pro Ala
290 295 300
Glu Lys Ile Val Ile Asn Phe Ile Thr Leu Asn Ile Ser Asp Asp Ser
305 310 315 320
Lys Ile Ser His Gln Asp Met Ser Leu Leu Gly Lys Ser Ser Asp Val
325 330 335
Ser Ser Leu Asn Asp Pro Gln Pro Ser Gly Asn Leu Arg Pro Pro Gln
340 345 350
Glu Glu Glu Glu Val Lys His Leu Gly Tyr Ala Ser His Leu Met Glu
355 360 365
Ile Phe Cys Asp Ser Glu Glu Asn Thr Glu Gly Thr Ser Phe Thr Gln
370 375 380
Gln Glu Ser Leu Ser Arg Thr Ile Pro Pro Asp Lys Thr Val Ile Glu
385 390 395 400
Tyr Glu Tyr Asp Val Arg Thr Thr Asp Ile Cys Ala Gly Pro Glu Glu
405 410 415

```
Gln Glu Leu Ser Leu Gln Glu Glu Val Ser Thr Gln Gly Thr Leu Leu
            420                 425                 430
Glu Ser Gln Ala Ala Leu Ala Val Leu Gly Pro Gln Thr Leu Gln Tyr
        435                 440                 445
Ser Tyr Thr Pro Gln Leu Gln Asp Leu Asp Pro Leu Ala Gln Glu His
    450                 455                 460
Thr Asp Ser Glu Glu Gly Pro Glu Glu Glu Pro Ser Thr Thr Leu Val
465                 470                 475                 480
Asp Trp Asp Pro Gln Thr Gly Arg Leu Cys Ile Pro Ser Leu Ser Ser
                485                 490                 495
Phe Asp Gln Asp Ser Glu Gly Cys Glu Pro Ser Glu Gly Asp Gly Leu
            500                 505                 510
Gly Glu Glu Gly Leu Leu Ser Arg Leu Tyr Glu Glu Pro Ala Pro Asp
        515                 520                 525
Arg Pro Pro Gly Glu Asn Glu Thr Tyr Leu Met Gln Phe Met Glu Glu
    530                 535                 540
Trp Gly Leu Tyr Val Gln Met Glu Asn
545                 550
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 451 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AGTGTGTGAC CAACCACACG CTGGTGCTCA CCTGGGCTGG AGCCGAACAC TCTTTACTGC     60
GTACACGTGG AGTCCTTCGT CCCAGGGCCC CCTCGCCGTG CTCAGCCTTC TGAGAAGCAG    120
TGTGCCAGGA CTTTGAAAGA TCAATCATCA GAGTTCAAGG CTAAAATCAT CTTCTGGTAT    180
GTTTTGCCCA TATCTATTAC CGTGTTTCTT TTTTCTGTGA TGGGCTATTC CATCTACCGA    240
TATATCCACG TTGGGCAAAG AGAAACACCC AGGCAAATTT GATTTTGATT TATGGGAAAT    300
GAATTTGACA AAAGATTCTT TGTGCCTGCT GGAAAAAATC GTGGATTAAC TTTATTCACC    360
CTCAATATCT CGGGTGGATT CTAAAATTTT CTCCATCCAG GGGTATGGAG GTTTACTGGG    420
GGTAAANGCG GGTGTTGTTT NCCAGGCCTT A                                    451
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC11107

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCTGGGTGTT TCTCTTTG 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCCTAAT ACGACTCACT ATAGGGC 27

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC11108

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAATAGCCC ATCACAGAAA 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACTCACTATA GGGCTCGAGC GGC 23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC6091

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAGCACAGAA TTCACTACTC GAGGCGGCCG CTTTTTTTTT TTTTTTTTT 49

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other
(vii) IMMEDIATE SOURCE:
(B) CLONE: ZC11526

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CACCAGAGGG TCTTCAAGGA GT 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(ix) FEATURE:

(A) NAME/KEY: Other
(B) LOCATION: 3...3
(D) OTHER INFORMATION: Xaa = any amino acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Trp Ser Xaa Trp Ser
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGATCCTTTG TGCCTGCTGA 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Other

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTATTGTTCT GCTGAGGGAC 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 221 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Val Pro Cys Val Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe
 1               5                  10                  15
Leu Ser Ile Asn Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu Gly
            20                  25                  30
Leu Gln Gly Val Lys Val Thr Tyr Thr Val Gln Tyr Phe Ile Tyr Gly
        35                  40                  45
Gln Lys Lys Trp Leu Asn Lys Ser Glu Cys Arg Asn Ile Asn Arg Thr
    50                  55                  60
Tyr Cys Asp Leu Ser Ala Glu Thr Ser Asp Tyr Glu His Gln Tyr Tyr
65                  70                  75                  80
Ala Lys Val Lys Ala Ile Trp Gly Thr Lys Cys Ser Lys Trp Ala Glu
                85                  90                  95
Ser Gly Arg Phe Tyr Pro Phe Leu Glu Thr Gln Ile Gly Pro Pro Glu
            100                 105                 110
Val Ala Leu Thr Thr Asp Glu Lys Ser Ile Ser Val Val Leu Thr Ala
        115                 120                 125
Pro Glu Lys Trp Lys Arg Asn Pro Glu Asp Leu Pro Val Ser Met Gln
    130                 135                 140
Gln Ile Tyr Ser Asn Leu Lys Tyr Asn Val Ser Val Leu Asn Thr Lys
145                 150                 155                 160
Ser Asn Arg Thr Trp Ser Gln Cys Val Thr Asn His Thr Leu Val Leu
                165                 170                 175
Thr Trp Leu Glu Pro Asn Thr Leu Tyr Cys Val His Val Glu Ser Phe
            180                 185                 190
Val Pro Gly Pro Pro Arg Arg Ala Gln Pro Ser Glu Lys Gln Cys Ala
        195                 200                 205
Arg Thr Leu Lys Asp Gln Ser Ser Glu Phe Lys Ala Lys
    210                 215                 220
```

CLAIMS

We claim:

1. An isolated polypeptide comprised of (a) a sequence of amino acid residues extending from residue 30, a valine, residue 250, a lysine, of SEQ ID NO: 2 (b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b),

2. The isolated polypeptide of claim 1 wherein the sequence of amino acid residues extends from residue 30, a valine, through residue 274, a tyrosine.

3. The isolated polypeptide of claim 1 wherein the sequence of amino acid residues extends form residue 30, a valine residue, through residue 553, an asparagine residue.

4. A polypeptide containing a subsequence of SEQ ID NO: 2 wherein said subsequence is at least 20 amino acids long.

5. A polypeptide of claim 1 further comprised of a transmembrane domain, an intracellular domain or both.

6. An isolated polynucleotide which encodes a polypeptide defined by claims 1-4.

7. An expression vector which contains the polynucleotide of claim 6 the following operably linked elements:

a transcription promoter;

a polynucleotide sequence of claim 5 and

a transcription terminator.

8. A method for producing an antibody comprising:

inoculating a polypeptide defined by SEQ ID NO: 2, a subsequence thereof or a polypeptide containing at least 20 amino acids of SEQ ID NO: 2 to an animal wherein said animal produces antibodies which bind to the polypeptide of SEQ ID NO: 2; and

isolating said antibodies.

9. An antibody produced by the process of claim 7.

10. An anti-idiotypic antibody which binds to and neutralizes an antibody of claim 9.

11. An antibody which specifically binds to a polypeptide of claims 1-4.

12. A chimeric polypeptide consisting essentially of a first portion and a second portion joined by a peptide bond, said first portion consisting essentially of a ligand binding domain of a receptor polypeptide selected from the group consisting of:

(a) a receptor polypeptide as shown in SEQ ID NO:2 containing residues 30-250;

(b) allelic variants of SEQ ID NO:2; and

(c) receptor polypeptides that are at least 80% identical to (a) or (b),

and said second portion consisting essentially of an affinity tag.

13. A polypeptide according to claim 12 wherein said affinity tag is an immunoglobulin F_C polypeptide.

14. A method for detecting a ligand within a test sample, comprising contacting a test sample with a polypeptide comprising a segment selected from the group consisting of:

(a) residues 30 to 250 of SEQ ID NO:2;

(b) allelic variants of (a); and

(c) sequences that are at least 80% identical to (a) or (b),

and detecting binding of said polypeptide to ligand in the sample.

15. A method according to claim 14 wherein said polypeptide further comprises transmembrane and intracellular domains.

16. A method according to claim 15 wherein said polypeptide is membrane bound within a cultured cell, and said detecting step comprises measuring a biological response in said cultured cell.

17. A method according to claim 16 wherein said biological response is cell proliferation or activation of transcription of a reporter gene.

18. A method according to claim 14 wherein said polypeptide is immobilized on a solid support.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/US 98/03029

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/715 C12N15/62 C07K16/28 C07K16/42
C07K19/00 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LUTFALLA G ET AL: "A new member of the cytokine receptor gene family maps on chromosome 21 at less than 35kb from IFNAR" GENOMICS, vol. 16, 1993, pages 366-373, XP002068797 cited in the application	
A	LUTFALLA G ET AL: "Structure of the human CRFB4 Gene: Comparison with its IFNAR neighbor" JOURNAL OF MOLECULAR EVOLUTION, vol. 41, 1995, pages 338-344, XP002068798	

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 1998	06/07/1998

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